

Amendments to the Claims:

The following amendments and cancellations are made without prejudice or disclaimer. This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

1. – 10. (cancelled)

11. (currently amended) A method of evaluating the deacetylation of a substrate in the presence of a Sir2 protein, NAD or an NAD-like compound and an agent, the method comprising:

a) combining a substrate that comprises [[an]] one or more acetylated amino acid side chains, an isolated or recombinantly produced Sir2 protein, NAD or an NAD-like compound and an agent to be tested, thereby producing a combination; and

b) determining if ~~the~~ an acetylated amino acid side chain in the substrate is deacetylated.

12. – 168. (canceled)

169. (previously presented) The method of claim 11 wherein step (b) comprises electron-spray mass spectroscopy.

170. (previously presented) The method of claim 11 further comprising comparing deacetylation of the substrate in the presence of the agent to deacetylation of the substrate in the absence of the agent, wherein a difference in substrate deacetylation indicates that the agent alters Sir2 protein deacetylase activity.

171. (previously presented) The method of claim 11 wherein the Sir2 protein is a human Sir2 protein.

172. (previously presented) The method of claim 11 wherein the Sir2 protein is a murine Sir2 protein.

173. (previously presented) The method of claim 11 wherein the Sir2 protein is a fusion protein.

174. (previously presented) The method of claim 11 wherein the substrate is a fragment of a histone that comprises the N-terminal tail of a histone protein.

175. (previously presented) The method of claim 174 wherein the histone protein is histone H3.

176. (previously presented) The method of claim 175 wherein the N-terminal tail of histone H3 is acetylated at lysine 9 and/or lysine 14.

177. (previously presented) The method of claim 11 wherein the substrate is a histone protein.

178. (previously presented) The method of claim 177 wherein the histone protein is selected from the group consisting of an H2B, H3 and H4 histone protein.

179. (previously presented) The method of claim 177 wherein the histone protein is acetylated on a lysine amino acid residue.

180. (previously presented) The method of claim 179 wherein the histone protein is histone H4 and the protein is acetylated on lysine 16 of histone H4.

181. (previously presented) The method of claim 11 wherein the acetylated amino acid is an acetylated lysine amino acid.

182. (previously presented) The method of claim 11 wherein the Sir2 protein is an isolated Sir2 protein.

183. (previously presented) The method of claim 11 wherein the Sir2 protein is a recombinantly produced Sir2 protein.

184. (previously presented) The method of claim 11 wherein the combination comprises  $\text{MgCl}_2$ .

185. (previously presented) The method of claim 11 wherein the combination comprises dithiothreitol (DTT).

186. (previously presented) The method of claim 11 further comprising formulating the agent with a pharmaceutically acceptable carrier to provide a pharmaceutical composition.

187. (previously presented) The method of claim 186 wherein the pharmaceutically acceptable carrier comprises a carbohydrate.

188. (previously presented) The method of claim 11 wherein the combination comprises NAD.

189. (previously presented) The method of claim 11 wherein the Sir2 protein is a Sir2 $\alpha$  protein.

190. (previously presented) The method of claim 189 wherein the Sir2 $\alpha$  protein comprises SEQ ID NO:12.

191. (previously presented) A method of evaluating the deacetylation of a substrate in the presence of a human Sir2 protein, NAD, and an agent, the method comprising:

a) providing a mixture comprising a substrate that comprises [[an]] one or more acetylated amino acid side chains, an isolated or recombinantly produced human Sir2 protein, NAD, and an agent to be tested; and

b) determining if ~~the~~ an acetylated amino acid side chain in the substrate is deacetylated.

192. (previously presented) The method of claim 191 wherein the mixture comprises MgCl<sub>2</sub>.

193. (previously presented) The method of claim 191 wherein the mixture comprises dithiothreitol (DTT).

194. (previously presented) The method of claim 11 or 191 wherein the SIR2 protein is produced in *E. coli*.

195. (previously presented) The method of claim 11 or 191 wherein the agent is a protein.

196. (previously presented) The method of claim 11 or 191 wherein the agent is a peptide.

197. (previously presented) The method of claim 11 or 191 wherein the agent is naturally occurring.

198. (previously presented) The method of claim 11 or 191 wherein the agent is non-naturally occurring.

199. (previously presented) The method of claim 11 or 191 wherein the agent is chemically synthesized.

200. (previously presented) The method of claim 11 or 191 wherein the agent is a carbohydrate.

201. (previously presented) The method of claim 11 or 191 wherein the agent is a steroid.

202. (previously presented) The method of claim 11 or 191 wherein the agent is a lipid.

203. (previously presented) The method of claim 11 or 191 wherein the agent is an anion.

204. (previously presented) The method of claim 11 or 191 wherein the agent is a cation.

205. (previously presented) The method of claim 11 or 191 wherein the agent is an oligonucleotide.

206. (previously presented) The method of claim 195 wherein the agent is an antibody.

207. (previously presented) The method of claim 191 wherein the Sir2 protein is an isolated Sir2 protein.

208. (previously presented) The method of claim 191 wherein the Sir2 protein is a recombinantly produced Sir2 protein.

209- 219. (cancelled)